The Media of Rapid Urease Test Influence the Diagnosis of *Helicobacter pylori*

Choo-Hung Kuo, Deng-Chyang Wu, Chien-Yu Lu, Yu-Chung Su, Fang-Jong Yu, Yi-Chen Lee I-Chen Wu, Shiu-Ru* Lira[2], *Chiang-Shin Liu[2], Chang-Ming Jan, Wen-Ming Wang

Departments of Internal Medicine, Microbiology, and Pathology, Division of Gastroenterology Kaohsiung Medical University Hospital, Kaohsiung, Taiwan

Corresponding Author: Dr. Deng-Chyang Wu, Endoscopy room, Kaohsiung Medical University Hospital 100 Shih-Chuan 1st Road, Kaohsiung, 807, Taiwan Tel: +886 7 3208158, Fax: +886 7 3135612, E-mail: dechwu@yahoo.com

ABSTRACT:

Background/Aims: The influence of different media on the validity of the rapid urease test, including accuracy, reaction time and cost-effectiveness is evaluated.

Methodology: Biopsies were obtained from the antral and body mucosa of 100 KMUH patients (51 men, 49 women; mean age: 54.0 years, range: 21-79 years old) undergoing gastroendoscopy due to dyspepsia. None of the patients had received any *Helicobacter pylori* eradicating treatment, nor any other antibiotic or bismuth treatment in the previous one month, nor had they had any type of gastric operation in the past. *Helicobacter pylori* status was evaluated by seven different tests: culture, histology, home-made rapid urease test, 13C-urea breath test, and three different commercially available rapid urease tests - including the CLOtest, the Pronto Dry test, and the Pyloriset Urease test. *Helicobacter pylori* status was denned as positive when the culture was positive or if concordance of two of the other three tests (histology, homemade rapid urease test and 13C-urea breath test) was positive.

Results: Three different rapid urease tests have similar sensitivities (97.3% us. 100% *vs.* 100%) and specificities (98.4% us. 96.8% us. 98.2%), and accuracy (98.4% us. 96.8% us. 98.2%). But the reaction time was longer in the CLOtest than for the other two rapid urease tests (22.3 us. 5.6 us. 10.1 minutes) ($P<0.05$). The Pronto Dry test and the Pyloriset Urease test also have more rapid positive rate than CLOtest. However, *in vitro* study, three tests show similar rapidity of positive reaction at different densities of *Helicobacter pylori.* ;

Conclusions: These three tests have practical advantages for physicians who need a rapid and accurate method of diagnosing *Helicobacter pylori* infections. The Pronto Dry test and Pyloriset Urease test have degrees of accuracy similar to the CLOtest, but results are obtained more rapidly and they are cheaper. Furthermore, the Pronto Dry test can be stored at room temperature and thereby save on the storage expense.

KEY WORDS: Rapid urease test. (RUT); *Helicobacter pylori {Hp),* Diagnosis

ABBREVIATIONS: Rapid Urease Test (RUT); *Helicobacter pylori (Hp):* Urea Breath Test (UBT); Mucosa-Associated Lymphoid Tissue Lymphoma (MALToma); Positive Predictive Value (PPV); Negative Predictive Value (NPV)

INTRODUCTION

Since the discovery of *Helicobacter pylori (Hp)* by Marshall and Warren in 1983 (1), overwhelming evidence has accumulated to confirm that *Hp* infection plays a significant role in the development of chronic active gastritis, peptic ulcer, and gastric adenocarcinoma (2-6). *Hp* infection is very common throughout the world, occurring in 40-50% of the population in developed countries and 80-90% of the population in developing regions (7), and about 54.4% of the people in Taiwan (8).

A large number of methods, most of which require gastric biopsies, have been used to diagnose *Hp* infection, but there is no single gold standard test for the diagnosis of *Hp* infection (9-11). Every method for detection of *Hp* has its own inherent advantages and disadvantages. Although biopsy based tests may suffer from sampling error (12) due to the patchy nature of the infection, rapid urease test (RUT), with its high sensitivity and specificity, is considered to be a quick and reliable test for the initial diagnosis of *Hp* infection. Also, RUT is simple and inexpensive (13-16). When a biopsy is incubated in a medium containing urea and a pH-sensitive color marker, urease hydrolyses urea to carbon dioxide and ammonia, causing a rise in pH value and a change in the color of the medium.

In the past, the reaction time of most RUTs required 30 minutes on average, may have needed more than 4 hours in some cases (17,18). Patients must know the RUT results before their next visit to the OPD. If the reaction time can be reduced to less than 10 minutes, it allows a diagnosis to be made before the patient leaves the endoscopy suite. This may save on medical expenses due to repeated visits to the clinic and reduce paper work. It may also increase the success rate of eradicating *Hp,* because compliance is considered a major factor in the successful treatment of *Hp,* compliance may improve significantly if we make an early diagnosis of *Hp* infection and initiate therapy (19).

The present prospective study was undertaken to evaluate the validity of RUTs containing different media. This evaluation included accuracy, reaction time and cost-effectiveness. We also wanted to evaluate the positive reaction of different RUTs in relation to various colonies of *Hp*, and the accuracy of different RUTs in patients who had received prior *Hp* eradication therapy.

METHODOLOGY

Subjects

This study included 100 patients (59 men and 41 women; mean age: 54.3 yr; range: 21-79 yr). All patients received gastroendoscopic examination due to dyspepsia; 31 non-ulcer dyspepsias, 65 peptic ulcers and 4 gastric malignancies (3 adenocarcinoma and 1 MALToma) were diagnosed. Exclusion criteria were the following: antibiotics, bismuth salts, or proton-pump inhibitor used during the previous one month, previous anti-*Hp* treatment; prior gastric surgery;
presence of a bleeding peptic ulcer; severe concomitant disease; and pregnancy or lactation.

Diagnostic Tests for *Hp* Infection

All participants received endoscopic examinations with two sets of biopsies (containing one specimen from the lesser curvature side of the antrum and another specimen from the greater curvature side of the gastric body). Histological examination was performed using one set of biopsy specimens that were fixed with formalin, embedded in paraffin and stained with hematoxylin and eosin. Culture of *Hp* was carried out on the second set of specimens, which was rubbed on the surface of a Campy-BAP agar plate [Brucella agar (Difco) + IsoVitalex (Gibco) + 10% whole sheep blood], and then incubated at 35 °C under microaerobic conditions (5% 02, 10% C02, and 85% N2) for 4-5 days. The culture of *Hp* was considered as positive if one or more colonies of gram-negative, oxidase (+), catalase (+), and urease (+) spiral or curved rods were presented. Besides these, three sets of biopsies (each one containing two antral specimens) were also obtained at a nearby area. One was tested by the CLOtest (Delta West Bentley, WA Australia), and the results of the CLOtest were interpreted as positive if the color of the gel changed from yellow to pink or red within 24 hours at room temperature. The reaction time was recorded in minutes. The other two sets were examined by Pronto Dry test (Medical Instruments Corp., Solothurn, Switzerland) and Pyloriset Urease test (Orion Corp. Orion Diagnostica, Espoo, Finland), respectively. The results of these tests were interpreted by the same procedure as the CLO-test. The media contained in the CLOtest, the Pronto Dry test and the Pyloriset Urease test was semi-solid phase (agar gel), solid phase (dry plate) and liquid phase (bottle), respectively. For "C-urea breath test (UBT), participants were asked to drink 100 mg of 99% isc-labeled urea in water during the fasting status (20). Breath samples were collected before and 15 min after administration of 13C-urea. The 13C02 in the breathing samples was analyzed using a continuous-flow isotope ratio mass spectrometer (CF-IRMS) (Europe Scientific Ltd, Crewe, U.K.). The cut-off value of 13C-UBT was 4.2 per mL at the 15 minutes after taking "C-urea,

Confirmation of *Hp* Infection

Hp infection was confirmed when the culture was positive or two of the other three tests (biopsy RUT, histology and 13C-UBT) were positive. If all four tests were negative, or if only one test of biopsy, RUT, histology or 13C-UBT was positive, this was interpreted as being negative and was excluded from this study.

The Rapidity of RUTs in Relation to the Number of *Hp*

Fresh *Hp agar* cultures of *Hp* strains (grown for 72 hours) were washed out with saline solution, centrifuged at 10000 rpm for 5 minutes and suspended in the new portion of saline solution. The *Hp* colony in this solution was estimated by means of McFarland nephelometer standards, and its final cell concentration was adjusted with saline to 10^9,10^8,10^7, 10^6, and 10^5 CFU per ml. 0.0l ml of each *Hp* solution was then transferred onto three different RUTs. The reaction time at room temperature was recorded in minutes.

Statistical Analysis

DX^2 & Fisher's exact tests were used for the statistical analysis

RESULTS

Data presented in **Table 1** show the results of three different RUTs that include sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), mean reaction time and price. It shows that three RUTs (CLOtest *us.* Pronto Dry test *vs.* Pyloriset Urease test) have similar sensitivities (97.3% us. 100% us. 100%), specificities (98.4% *us.* 96.8% us. 98.2%) and accuracy (98.4% *vs.* 96.8% us. 98.2%). However, the CLOtest has a longer mean reaction time than the other two tests (22.3 us. 5.6 us. 10.1 minutes) ($P<0.05$), and seems to be more expensive than the other two.

Figure 1 shows that more than 90% of *Hp* infected patients show positive results within 10 minutes for both the Pronto Dry test and the Pyloriset Urease test, but the CLOtest requires more than 30 minutes to get the same percentage. However, up to 50 minutes, all three tests have shown the same degrees of positive rate. Results can be known rapidly and accurately (even within 10 minutes) for most patients who received RUT using the Pronto Dry test and the Pyloriset Urease test,

TABLE 1 The Validity of Three Commercially Available Rapid Urease Tests

Method	Case No.	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Mean PRT (minute)	Price
CLOtest	100	97.3	98.4	97.3	98.4	22.3**	$4.41
Pronto Dry test	100	100	96.8	95	100	5.6*	$3.53
Pyloriset Urease test	90	100	98.2	97.0	100	*10.1**	$2.94

PPV: positive predictive value; NPV: negative predictive value; PRT: positive reaction time. *: <P<0.05.

rather than CLOtest. Most patients received the Pronto Dry test or the Pyloriset Urease test during gastroendoscopic examination can obtain their *Hp* infection results before leaving the office.

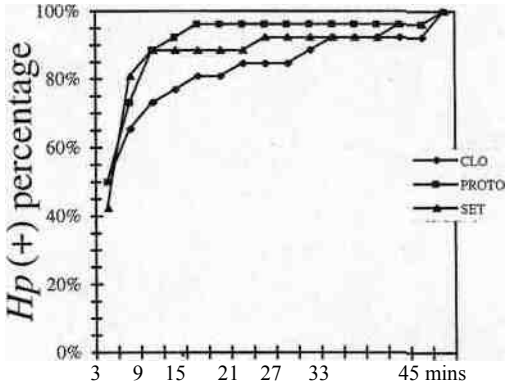
Figure 2 shows that we compared the reaction time of different RUTs with various densities of *Hp in vitro.* The lowest density, which the tests determine as positive, is 10^6 CFU per ml, and the reaction time is about 240 minutes. Three RUTs have similar reaction times in various densities of *Hp* when the density is more than 10^6 CFU per ml.

DISCUSSION

There is still no well-accepted "golden standard" for diagnosis of *Hp* infection. The non-invasive tests seem to be more acceptable by patients than the invasive tests; consequently, recent studies almost always focus on non-invasive tests (21). However, there is no statistical difference between these two groups (22,23). Besides this, biopsy-based methods are essential for the initial diagnosis of *Hp* for patients undergoing endoscopic examination. RUTs are the method of choice for detecting *Hp* in patients undergoing endoscopy, because they are easily performed, inexpensive and reliable (15,24,25). RUTs also do not require other complex facilities that are needed for culture, histology, 13C-UBT and other non-invasive tests. Furthermore, other tests are time consuming, but RUTs can quickly diagnose *Hp* infections, enabling the physician to initiate therapy for patients. Also, RUTs could save medical expenses from repeat visits to the clinic and reduce paper work. Because of this, rapid urease tests are more practical and suitable for most gastroenterologists, whether they work in a hospital or a clinic.

The CLOtest was the main standard for rapid urease tests in the past. It has been reported to have a sensitivity of 89.6%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 84.1% (16). Our data has shown similar results: sensitivity (97.3%), specificity (98.4%), PPV (97.3%) and NPV (98.4%). All three RUTs have similar degrees of accuracy in our study, so the media used in RUTs may not affect the accuracy of tests. However reaction time needed for the CLOtest is longer than the other two tests. This may be due to the fact that reagents in the CLOtest are contained in an agar gel, and therefore a relatively longer time is needed both for diffusion of urease and a clearly positive result. In a previous study, the rapidity of which the CLOtests turns positive was reported to be 75% in 20 minutes, 85% by 1 hour, 92% by 3 hours and 98% by 24 hours (18). In our study, the rapidity of which the CLOtests turns positive was 75% in 20 minutes, 90% by 1 hour, 95% by 3 hours and 99% by 24 hours. The rapidity of which the Pronto Dry and the Pyloriset Urease tests turn positive was 92% and 88%, respectively in 20 minutes, 98% and 97%, respectively by 1 hour and 100% by 24 hours. This shows that results can be known rapidly and accurately (even within as little as 10 minutes) for most patients who receive RUTs using the Pronto Dry and Pyloriset Urease tests, rather than the CLOtest.

In ire *vitro* studies, we found that the lower the density of *Hp,* the longer the reaction time needed. Again, in *in vitro* studies, all three RUTs revealed negative results when the density of *Hp is* less than 10^5 CFU per ml. We did not find significant differences, of reaction times for the different RUTs in different densities *of Hp. So* we assume that both the density of *Hp* and the diffusing time of urease from specimen to medium might influence the positive reaction time of RUTs. Accordingly, we may get more rapid and accurate results if we use a media suitable for the diffusion of urease.



CLO: CLOtest; PRONTO: Pronto Dry test; SET: Pyloriset Urease test.

FIGURE 1 Comparison of the *Hp*-positive percentage rates at different reaction times in *Hp* infected patients among three commercially available rapid urease tests.

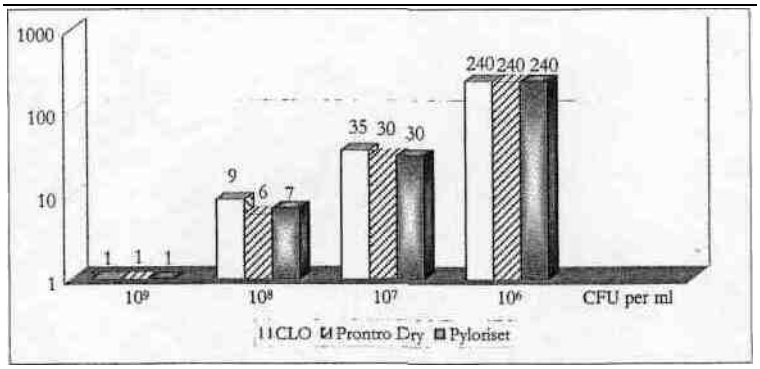


FIGURE 2 The rapidity of RUTs in relation to *Hp* density in *in vitro* tests.

Of these three RUTs, the Pronto Dry test also has the advantage in that it need not be refrigerated which is the case with the other two tests. One disadvantage of the PyloriSet urease test is that the result is more difficult to be interpreted when the color change is only slight.

The possibility of gastric mucosa atrophy and intestinal metaplasia becomes greater in older patients, which reduces the sensitivity of the biopsy-based methods (26), so a greater number of patients may need to be evaluated to determine whether the media may influence the result of these tests or not. A lower accuracy of RUT in bleeding peptic ulcer was

reported in previous studies (27-29), but the sensitivities of UBT, culture, histology and serology were not affected by blood in the antrum (30,31). Thus, further studies may be needed decide if the improvement of accuracy in bleeding peptic ulcer occurs when using different media.

CONCLUSIONS

Although the non-invasive tests of *Hp* is gradually well accepted by patients, RUTs is still the first choice for initial diagnosis of *Hp* infection. The solid medium, which is used in the Pronto Dry test is simpler to prepare, results are available more rapidly and it is cheaper. If the reaction time of RUTs was shorter, they may be more cost-effective and can save medical expenses incurred by repeated clinical visits, and also reduce the amount of paper work necessary. Next step of this study should focus on improvement of accuracy in bleeding peptic ulcer and aging people by simply changing the test media.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Science Council of the ROC (NSC-90-2314-B-037-104) and Kaohsiung Medical University (KMU-91-B-02). The authors would thank the support and assistant from the Statistic unit of Department of Clinical Research in KMUH.

REFERENCES

1 **Marshall B:** Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983; 1:1273-1275.

2 **International Agency for Research on Cancer WHO:** Infection with *Helicobacter pylori.* In: Schistosomes, liver ' flukes and *Helicobacter pylori.* Lyon: IARC 1994; pp. 177-202.

3 **Goodwin** CS, **Armstrong JA, Marshall BJ:** *Campylobac-ter pyloridis* gastritis and peptic ulceration. J Clin Microbiol 1986; 39:353-356.

4 **Rauws EAJ, Tytgat GNJ:** *Campylobacter pyloridis:* treatment of gastritis. In: Bathbone BJ, Heatley RV (eds.). *Campylobacter pyloridis* and Gastroduodenal Disease. Oxford: Black-Well, 1989; pp. 225-236.

5 **Wyatt JI, Rathbone BJ, Dixon MF, Heatley RV:** *Campylobacter pyloridis* and acid induced gastric metaplasia in the pathogenesis of duodenitis. J Clin Pathol 1987; 40:847-848.

6 **Hsu CT, Yeh C, Cheng HH:** *Helicobacter pylori,* gastritis and duodenitis in the healing process of duodenal ulcer. J For-mosan Med Assoc 1992; 91:81-84.

7 **Vaira D, Miglioli M, Mule P, Holton J, Menegatti M, Vergura** M, **et al:** Prevalence of peptic ulcer in *Helicobacter pylori* positive blood donors. Gut 1994; 35:309-312.

8 **Lin JT, Wang JT, Wang TH, Wu MS, Lee TK, Chen CJ:** *Helicobacter pylori* infection in a randomly selected population, healthy volunteers, and patients with gastric ulcer and gastric adenocarcinoma. A seroprevalence study in Taiwan. Scand J Gastroenterology 1993; 28:1067-1072.

9 **Dill** S, **Payne-James JJ, Misiewicz JJ, Grimble GK, McSwiggan D, Pathak K, Wood AJ, Scrimgeour CM, Rennie MJ:** Evaluation of 13C-urea breath test in the detection of *Helicobacter pylori* and in monitoring the effect of tripotassium dicitratobismuthate in non-ulcer dyspepsia. Gut 1990; 31:1237-1241.

10 **Kolts BE, Joseph B, Achem SR, Bianchi T, Monteiro C:** *Helicobacter pylori* detection: a quality and cost analysis. Am J Gastroenterol 1993; 88:650-655.

11 **Whitehead R, Truelove** SC, **Gear MWL:** The histological diagnosis of chronic gastritis in fiberoptic gastroscope biopsy specimens. J Clin Pathol 1972; 25:1-11.

12 **Van Zwet** AA, **Thijs JC, Roosendaal R, Kuipers EJ, Pena S, de GraaffJ:** Practical diagnosis *of Helicobacter pylori* infection. Eur J Gastroenterol Hepatol 1996; 8(5):501-507.

13 **El-Zimaity H/MT, Al-Assi MT, Genta RM, Graham DY:** Confirmation of successful therapy of *Helicobacter pylori* infection: number and site of biopsies or a rapid urease test. Am J Gastroenterol 1995; 90:1962-1964.

14 **Marshall BJ, Warren JR, Francis GJ, Langton SR, Goodwin CS, Blincow ED:** Rapid urease test in the management of *Campylobacter pylori* associated gastritis. Am J Gastroenterol. 1987; 82:200-210.

15 **Cohen H, Laine L:** Endoscopic methods for the diagnosis of *Helicobacter pylori.* Aliment Pharmacol Ther 1997; IKSuppI 1):3-9.

16 **Cutler A, Havstad** S, **Ma C,** et **al:** Accuracy of invasive and non-invasive tests to diagnose *Helicobacter pylori* infection. Gastroenterology 1995; 109:136-141.

17 **KatsuragiK,NodaA,TachikawaT,AzumaA,MukaiF, Murakami K, et al:** Highly sensitive urine-based enzyme-linked immunosorbent assay for detection of antibody to *Helicobacter pylori.* Helicobacter 1998; 3:289-295.

18 **Peura D:** *Helicobacter pylori:* a diagnostic dilemma and a dilemma of diagnosis. Gastroenterology 1995; 109:313-315

19 **Graham DY, Lew GM, Malaty HM, et al:** Factors influencing the eradication of*Helicobacterpylori* with triple therapy. Gastroenterology 1992; 102:493-496.

20 **Wang WM, Lee SC, Ding HJ, Jan CM, Chen LT, Wu DC:** Quantification of *Helicobacter pylori* infection: simple and rapid 13C-urea breath test in Taiwan. J Gastroenterol 1998; 33:330-335.

21 **Wu DC, Kuo CH, Lu CY, Jan CM, Wang WM, et al:** Evaluation of an office-based urine test for detecting *Helicobacter pylori:* a prospective pilot study. Hepatogastroenterology 2001; 48:614-617.

22 **Cutler AF, Havstad S, Chen KM, Blaser MJ, Perez-Perez GI, Schubert TT:** Accuracy of invasive and noninva-sive tests to diagnose *Helicobacter pylori* infection. Gastroenterology 1995; 109:136-141.

23 **Lin SK, Lambert JR, Schembri M, Nicholson L, Finlay M, Wong C, et al:** A comparison of diagnostic tests to determine *Helicobacter pylori* infection. J Gastroenterol Hepatol 1992; 7:203-209.

24 **Hunt RH, Malfertheiner P, Yeomans ND, Hawkey CJ, Howden CW:** Critical issues in the pathophysiology and management of peptic ulcer disease. Eur J Gastroenterol Hepatol 1995; 7:685-699. /

25 **Elister Y, HiU I, Uchtman** SN, **Allan J:** Rosenberg. Prospective comparison of rapid urease tests (PyloriTek, CLOtest) for the diagnosis of'*Helicobacter pylori* infection in symptomatic children: a pediatric multicenter study. Am J of Gastroenterol 1998; 93:217-219.

26 **Atherton JC:** Non-endoscopic tests in the diagnosis *ot Helicobacter pylori* infection. Aliment Pharmacol Ther 1997; IKSuppI 1):11-20.

27 **Lee CL, Tu TC, Yang RN, et al:** Does blood in the stomach influence the diagnosis of *Hp* infection in patients with bleeding peptic ulcer? Gut 1997; 41(Suppl 1):A76.

28 **Leung WK, Sung JJ, Siu KL, Chan FK, Ling TK, Cheng AF:** False-negative biopsy urease test in bleeding ulcers caused by the buffering effects of blood. Am J Gastroenterol 1998; 93: 1914-1918.

29 **Tu TC, Lee CL, Wu CH,** et **al:** Comparison of invasive and noninvasive tests for detecting *Helicobacter pylori* infection in bleeding peptic ulcers. Gastrointest Endosc 1999; 49:302-306.

30 **Archimandritis A, Tzivras M, Souyioultzis S, Papaparaskevas I, Apostolopulos P, Delladetsima I et al;** High rates of false negative rapid urease test (CLO) in patients with upper gastrointestinal bleeding (UGB). Gut 1997; 41(1 Suppl):A76.

31 **Lai KC, Hui WM, Lam SK:** Bleeding ulcers have high false negative rate for antral *Helicobacter pylori* when tested with urease test. Gastroenterology 1996; 110:Al67.